
Sequence Listing could not be accepted due to errors.

See attached Validation Report.

If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).

Reviewer: Anne Corrigan

Timestamp: [year=2008; month=1; day=23; hr=13; min=45; sec=43; ms=375;]

Reviewer Comments:

<120> A METHOD FOR CREATING A FUNCTIONALLY ACTIVE CHIMERIC TYPE IIG RESTRICTION ENDONUCLEASE (as amended)

The first line of the above <120> response exceeds the Sequence Rules' required 72-character line (this includes white spaces). Please insert a hard return after "TYPE" on the first line, and align the second line.

(from Sequence 2)

Val Asp Glu Ala Leu Leu Ile Lys Tyr His Gly Phe Ser Glu Lys Glu 515 520 525

Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn 530 535 540

Asn Arg Thr Lys Lys 545

Please delete the excess blank lines above: only one blank line should separate each amino acid line.

<210> 38

<211> 103

<212> PRT

<213> artificial

<223>	segment	of.	protei	n seq	uence	of	cate	echol	0-methy	ltr	ansfe	erase	
<400>	39												
Please	change	the	above	<210>	respo	nse	to	"39."	<210>	38	was	alread	У

shown.

<220>

Validated By CRFValidator v 1.0.3

Application No: 10800946 Version No: 2.0

Input Set:

Output Set:

Started: 2008-01-09 14:09:09.990
Finished: 2008-01-09 14:09:12.054

Elapsed: 0 hr(s) 0 min(s) 2 sec(s) 64 ms

Total Warnings: 25
Total Errors: 2
No. of SeqIDs Defined: 43
Actual SeqID Count: 43

Error code		Error Descript	ion								
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(19)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(20)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(21)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(22)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(23)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(24)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(25)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(26)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(27)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(28)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(29)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(30)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(31)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(32)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(33)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(34)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(35)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(36)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(37)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(38)

Input Set:

Output Set:

Started: 2008-01-09 14:09:09.990 Finished: 2008-01-09 14:09:12.054

Elapsed: 0 hr(s) 0 min(s) 2 sec(s) 64 ms

Total Warnings: 25
Total Errors: 2

No. of SeqIDs Defined: 43

Actual SeqID Count: 43

	Erro	r code	Error Description
			This error has occured more than 20 times, will not be displayed
1	Ξ	212	Invalid Sequence ID Number; Expected 39 as next SeqID but skipped
1	Ξ	212	Invalid Sequence ID Number; Expected 39 as next SeqID but skipped

```
<110> Xu, Shuang-yong
      Kobbe, Daniela
       Zhu. Zhenvu
       Samuelson, James
<120> A METHOD FOR CREATING A FUNCTIONALLY ACTIVE CHIMERIC TYPE IIG RESTRICTION ENDONUCLEASE
(as amended)
<130> NEB-183-CIP
<140> 10800946
<141> 2004-03-15
<150> 10/150.028
<151> 2002-05-17
<150> 09/693.146
<151> 2000-07-02
<160> 43
<170> PatentIn version 3.2 (1-26) and 3.4 (27-43)
<210> 1
<211> 1650
<212> DNA
<213> Bacillus pumilus
<220>
<221> CDS
<222> (1)..(1650)
<400> 1
atg aat caa tta att gaa aat gtt aat cta caa aaa tta agg ggt ggg
Met Asn Gln Leu Ile Glu Asn Val Asn Leu Gln Lys Leu Arg Gly Gly
                5
                                   10
tat tac acc cct aaa gtt att gct gac ttt tta tgt caa tgg agt att
Tyr Tyr Thr Pro Lys Val Ile Ala Asp Phe Leu Cys Gln Trp Ser Ile
             20
                                25
caa gat gac aca aag agt gta ctt gaa ccc agt tgt gga gat ggt aat
Gln Asp Asp Thr Lys Ser Val Leu Glu Pro Ser Cys Gly Asp Gly Asn
         35
ttt att gaa tog goa ata ott agg tto aaa gaa ott agt ata gat aat
                                                                192
Phe Ile Glu Ser Ala Ile Leu Arg Phe Lys Glu Leu Ser Ile Asp Asn
                         55
gaa caa ctt aaa gga aga att aca gga gta gag cta att gaa gaa gaa
Glu Gln Leu Lys Gly Arg Ile Thr Gly Val Glu Leu Ile Glu Glu Glu
                    70
65
                                       75
                                                           8.0
gct ttg aaa gtt caa aat cga gca aat gag ttg ggg gtt gat aaa aac
```

Ala Leu Lys Val Gln Asn Arg Ala Asn Glu Leu Gly Val Asp Lys Asn

85 90 95

tca	ata	gta	aat	agt	gac	ttc	ttt	caa	ttt	gta	aaa	gat	aat	aag	aat	336
Ser	Ile	Val	Asn	Ser	Asp	Phe	Phe	Gln	Phe	Va1	Lys	Asp	Asn	Lys	Asn	
			100					105					110			
aaa	aaa	ttt	gat	act	att	att	ggt	aat	cca	cca	ttc	ata	aga	tac	caa	384
Lys	Lys	Phe	$_{\rm Asp}$	Thr	I1e	Ile	Gly	Asn	Pro	Pro	Phe	Ile	Arg	${\tt Tyr}$	Gln	
		115					120					125				
aac	ttt	cct	gaa	gag	cat	cgt	agt	ata	gcc	atg	gaa	atg	atg	gag	gaa	432
Asn		Pro	Glu	Glu	His		Ser	Ile	Ala	Met		Met	Met	Glu	Glu	
	130					135					140					
					aat											480
	Gly	Leu	Lys	Pro	Asn	Lys	Leu	Thr	Asn		Trp	Val	Pro	Phe		
145					150					155					160	
					tta											528
Val	Val	Ser	Ala		Leu	Leu	Asn	Glu		Gly	Lys	Met	Ala		Val	
				165					170					175		
					ttt											576
Ile	Pro	Ala		Leu	Phe	Gln	Val		Tyr	Ala	Ala	Glu		Arg	Ile	
			180					185					190			
					ttc											624
Pne	Leu	195	rās	Phe	Phe	Asp	Arg 200	IIe	Inr	ile	IIe	205	Pne	GIU	rās	
		195					200					205				
					atc											672
					Ile											672
Ten	210	Pile	GIU	ASII	TTe	215	GIII	GIU	Vall	ire	220	Leu	Leu	Cys	GIU	
	210					213					220					
2 20		a++	t		ggt		aas	2++	aaa	att	***	~~~	+ 00	ana	220	720
					Gly											120
225	210	,		2,0	230	2,0	012		1119	235			0,10	o.u	240	
										200						
tta	gat	gga	tta	aat	tcc	att	gat	ttt	gta	act	ata	aat	aat	t.ca	aat	768
					Ser											
				245					250					255		
qtt	aaa	cct	att	gaa	cac	cqt	act	qaa	aaq	taa	aca	aaq	tat	ttc	tta	816
					His											
			260					265	-			-	270			
aac	gaa	gat	gaa	ata	ctt	ctt	tta	cag	agt	tta	aag	gaa	gac	aaa	cgc	864
Asn	Glu	Asp	Glu	Ile	Leu	Leu	Leu	Gln	Ser	Leu	Lys	Glu	Asp	Lys	Arg	
		275					280					285				
gtt	aaa	aat	tgt	aat	gac	tat	ttt	aag	aca	gaa	gtt	ggc	tta	gtt	act	912
Val	Lys	Asn	Cys	Asn	Asp	Tyr	Phe	Lys	Thr	Glu	Val	Gly	Leu	Val	Thr	
	290					295					300					
gga	cga	aac	gaa	ttc	ttt	atg	atg	aaa	gaa	aac	caa	gta	aaa	gaa	tgg	960

Gly Arg Acn Glu Phe Phe Met Met Lys Glu Asn Gln Val Lys Glu Trp

305 310 315 320

	cta															1008
Asn	Leu	Glu	Glu		Thr	Ile	Pro	Val		Gly	Arg	Ser	Asn		Leu	
				325					330					335		
aaa	ggt	ata	aca	ttt	aca	gaa	aat	gat	ttt	cat	gaa	aat	tca	atg	gaa	1056
Lys	Gly	Ile	Thr	Phe	Thr	Glu	Asn	Asp	Phe	His	Glu	Asn	Ser	Met	Glu	
			340					345					350			
caa	aag	aca	att	cac	cta	ttt	tta	cca	cca	gat	gaa	gat	ttt	gaa	aaq	1104
	Lys															
		355					360					365				
	ccg															1152
Leu	Pro	Ile	Glu	Cys	Gln		Tyr	Ile	Lys	Tyr		Glu	Glu	Lys	Gly	
	370					375					380					
ttc	cat	caa	ggc	tat	aaa	acc	aga	att	aga	aaa	cgt	t gg	tat	ata	act	1200
Phe	His	Gln	Gly	Tyr	Lys	Thr	Arg	Ile	Arg	Lys	Arg	Trp	Tyr	Ile	Thr	
385					390					395					400	
	tet															1248
Pro	Ser	Arg	Trp	405	Pro	Asp	Ala	Pne	410	Leu	Arg	GIN	Val	415	GIY	
				405					410					415		
tat	cca	aaa	cta	att	tta	aat	gaa	acc	gac	gct	tct	tct	act	gat	aca	1296
Tyr	Pro	Lys	Leu	Ile	Leu	Asn	Glu	Thr	Asp	Ala	Ser	Ser	Thr	Asp	Thr	
			420					425					430			
att	cat	agg	qtt	aqa	ttt	aaa	qaa	qqt	ata	aat	gaa	aaq	tta	qcc	qta	1344
	His															
		435					440					445				
	tca															1392
val	450	File	Leu	Maii	Ser	455	TIIL	rile	мта	Ser	460	GIU	TTG	THE	GIY	
	4,70					4,,,					400					
aga	agt	tat	ggt	ggt	ggt	gtt	atg	aca	ttc	gaa	cca	act	gaa	att	gga	1440
-	Ser	Tyr	Gly	Gly	_	Val	Met	Thr	Phe		Pro	Thr	Glu	Ile	_	
465					470					475					480	
gaa	atc	cta	ata	cct	tcc	ttt	gat	aac	tta	tcc	att	gat	ttt	qat	aaa	1488
	Ile															
				485			-		490			-		495	-	
	gat															1536
Ile	Asp	Ala		Ile	Arg	Glu	Lys		Ile	Glu	Lys	Val		Asp	Ile	
			500					505					510			
gtt	gat	gaa	get	tta	ctt	ata	aaa	tat	cat	ggg	ttt	agt	gag	aaa	gaa	1584
	Asp															
		515					520					525				

gta aaa cag ctt cga ggg ata tgg aag aaa ctt tct cag aga aga aac $\,$ 1632 Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn

530 535 540

1650 aat aga acg aag aaa taa Asn Arg Thr Lys Lys 550 <210> 2 <211> 549 <212> PRT <213> Bacillus pumilus Met Asn Gln Leu Ile Glu Asn Val Asn Leu Gln Lys Leu Arg Gly Gly 10 Tyr Tyr Thr Pro Lys Val Ile Ala Asp Phe Leu Cys Gln Trp Ser Ile 2.0 25 Gln Asp Asp Thr Lys Ser Val Leu Glu Pro Ser Cys Gly Asp Gly Asn 40 Phe Ile Glu Ser Ala Ile Leu Arg Phe Lys Glu Leu Ser Ile Asp Asn Glu Gln Leu Lys Gly Arg Ile Thr Gly Val Glu Leu Ile Glu Glu Glu 65 70 75 Ala Leu Lys Val Gln Asn Arg Ala Asn Glu Leu Gly Val Asp Lys Asn 85 90 95 Ser Ile Val Asn Ser Asp Phe Phe Gln Phe Val Lys Asp Asn Lys Asn 100 105 Lys Lys Phe Asp Thr Ile Ile Gly Asn Pro Pro Phe Ile Arg Tyr Gln 115 120 Asn Phe Pro Glu Glu His Arg Ser Ile Ala Met Glu Met Met Glu Glu 135 Leu Gly Leu Lys Pro Asn Lys Leu Thr Asn Ile Trp Val Pro Phe Leu 150 155 Val Val Ser Ala Thr Leu Leu Asn Glu Gln Gly Lys Met Ala Met Val 165 170 175 Ile Pro Ala Glu Leu Phe Gln Val Lys Tyr Ala Ala Glu Thr Arg Ile 180 185 Phe Leu Ser Lys Phe Phe Asp Arg Ile Thr Ile Ile Thr Phe Glu Lys 195 200 Leu Val Phe Glu Asn Ile Gln Gln Glu Val Ile Leu Leu Cvs Glu 210 215

Lys Lys Val Asn Lys Gly Lys Gly Ile Arg Val Ile Glu Cys Glu Asn

225 230 235 240

Leu	Asp	Gly	Leu	Asn 245	Ser	Ile	Asp	Phe	Val 250	Ala	Ile	Asn	Gly	Ser 255	Asr
Val	Lys	Pro	11e 260	Glu	His	Arg	Thr	Glu 265	Lys	Trp	Thr	Lys	Tyr 270	Phe	Leu
Asn	Glu	Asp 275	Glu	Ile	Leu	Leu	Leu 280	Gln	Ser	Leu	Lys	Glu 285	Asp	Lys	Arç
Val	Lys 290	Asn	Суз	Asn	Asp	Tyr 295	Phe	Lys	Thr	Glu	Val 300	Gly	Leu	Val	Thi
Gly 305	Arg	Asn	Glu	Phe	Phe 310	Met	Met	Lys	Glu	Asn 315	Gln	Val	Lys	Glu	320
Asn	Leu	Glu	Glu	Tyr 325	Thr	Ile	Pro	Val	Thr 330	Gly	Arg	Ser	Asn	Gln 335	Leu
Lys	Gly	Ile	Thr 340	Phe	Thr	Glu	Asn	Asp 345	Phe	His	Glu	Asn	Ser 350	Met	Glu
Gln	Lys	Ala 355	Ile	His	Leu	Phe	Leu 360	Pro	Pro	Asp	Glu	Asp 365	Phe	Glu	Lys
Leu	Pro 370	Ile	Glu	Cys	Gln	Asn 375	Tyr	Ile	Lys	Tyr	Gly 380	Glu	Glu	Lys	Gly
Phe 385	His	Gln	Gly	Tyr	190 390	Thr	Arg	Ile	Arg	Lys 395	Arg	Trp	Tyr	Ile	Thr 400
Pro	Ser	Arg	Trp	Val 405	Pro	Asp	Ala	Phe	Ala 410	Leu	Arg	Gln	Val	Asp 415	Gly
Tyr	Pro	Lys	Leu 420	Ile	Leu	Asn	Glu	Thr 425	Asp	Ala	Ser	Ser	Thr 430	Asp	Thr
Ile	His	Arg 435	Val	Arg	Phe	Lys	Glu 440	Gly	Ile	Asn	Glu	Lys 445	Leu	Ala	Val
Val	Ser 450	Phe	Leu	Asn	Ser	Leu 455	Thr	Phe	Ala	Ser	Ser 460	Glu	Ile	Thr	Gly
Arg 465	Ser	Tyr	Gly	Gly	Gly 470	Val	Met	Thr	Phe	Glu 475	Pro	Thr	Glu	Ile	Gly 480
Glu	Ile	Leu	Ile	Pro 485	Ser	Phe	Asp	Asn	Leu 490	Ser	Ile	Asp	Phe	Asp 495	Lys
Ile	Asp	Ala	Leu 500	Ile	Arg	Glu	Lys	Glu 505	Ile	Glu	Lys	Val	Leu 510	Asp	Il∈
Val	Asp	Glu 515	Ala	Leu	Leu	Ile	Lys 520	Tyr	His	Gly	Phe	Ser 525	Glu	Lys	Glu

```
Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn
    530
                     535
                                           540
Asn Arg Thr Lys Lys
545
<210> 3
<211> 3030
<212> DNA
<213> Bacillus pumilus
<220>
<221> CDS
<222> (1)..(3030)
<400> 3
atq cat ata agt gag tta gta gat aaa tac aaa gcg cat aga agt act
Met His Ile Ser Glu Leu Val Asp Lys Tyr Lys Ala His Arg Ser Thr
1
ttt tta aaa cca act tat aat qaa act caa cta aqq aat qat ttt ata
Phe Leu Lys Pro Thr Tyr Asn Glu Thr Gln Leu Arg Asn Asp Phe Ile
             20
                                 25
gac cca ctt cta aaa tct tta gga tgg gat gtt gat aat acc aaa gga
                                                                  1/1/1
Asp Pro Leu Leu Lys Ser Leu Gly Trp Asp Val Asp Asn Thr Lys Gly
         35
                             40
aaa aca cat att cta aga gat gtc att caa gaa gaa tac ata gaa ata
                                                                  192
Lys Thr His Ile Leu Arg Asp Val Ile Gln Glu Glu Tyr Ile Glu Ile
                         55
aaa gat gag gag aca aag aaa aat cca gat tat aca ctt cgt ata aac
Lys Asp Glu Glu Thr Lys Lys Asn Pro Asp Tyr Thr Leu Arg Ile Asn
65
                     70
                                         75
                                                             80
ggt acg aga aag ctg ttt gta gag gtt aag aaa ccg tct ttt aat att
Gly Thr Arg Lys Leu Phe Val Glu Val Lys Lys Pro Ser Phe Asn Ile
                 85
                                     90
ttg aaa tca gct aaa gca gcc ttc caa aca aga aga tat ggt tgg agt
                                                                  336
Leu Lys Ser Ala Lys Ala Ala Phe Gln Thr Arg Arg Tyr Gly Trp Ser
            100
                               105
                                                    110
get aac ett ggt att tea gta ett aca aat tte gag eat eta gtt att
                                                                  384
Ala Asn Leu Gly Ile Ser Val Leu Thr Asn Phe Glu His Leu Val Ile
       115
                           120
tat gat tgt aga tat acq cct gac aaa tcc gac aat gaa cat att gct
Tyr Asp Cys Arg Tyr Thr Pro Asp Lys Ser Asp Asn Glu His Ile Ala
    130
                        135
                                            140
```

aga tat aaa git ito ici tac gag gaa tai gaa gaa gca iii gai gaa

Arg 145	Tyr	Lys	Val	Phe	Ser 150	Tyr	Glu	Glu	Tyr	Glu 155	Glu	Ala	Phe	Asp	Glu 160	
					tca Ser											528
				gta	aat Asn				ggt					gag		576
			180		gag			185					190			624
					Glu											
					gaa Glu											672
					aga Arg 230											720
					gaa Glu											768
					caa Gln											816
					gat Asp											864
					att Ile											912
					gtc Val 310											960
					gaa Glu											1008
					gaa Glu											1056
					gaa Glu											1104
					ttt										gat	1152

Thr Glu Gly Lys Lys Phe Asn Glu Leu Cys Asn Leu Lys Ile Ala Asp

370 375 380

ata tgt tgt gga tca gga act ttc cta att tca agt tat gac ttt cta 1200 Ile Cys Cys Gly Ser Gly Thr Phe Leu Ile Ser Ser Tyr Amp Phe Leu 385 390 195 400